

Integral use of prickly pear xoconostle fruits to obtain bioactive compounds using a biorefinery approach

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Abstract: The prickly pear xoconostle (*Opuntia matudae*) currently lacks industrial applications, as its use is mainly focused on traditional products such as beverages, sauces, and confections. To enhance the value of this crop in producing regions, this study evaluated the extraction of bioactive compounds with potential applications in the biotechnology and food industries. The main objective of this study was the extraction of pectin from the fruit peel, through acid hydrolysis. The process comprised the following stages: grinding, ethanol washing, extract recovery, pectin precipitation, and drying, resulting in a yield of 9.95% of pectin. The washing effluents were analyzed for betalain content, yielding a concentration of 2.021 g/mL as total betalains. This liquid phase was subsequently subjected to distillation; a 70% yield of ethanol was obtained with 65% purity. The solid residue obtained from the extraction was further utilized for crude fiber isolation, achieving a yield of $72.3 \pm 3.48\%$. These findings demonstrate that xoconostle fruits offer a promising raw material for the extraction of pectins, betalains, and crude fiber, and the proposed recycling of ethanol and water, supports the development of a sustainable process in a biorefinery.

Keywords: xoconostle, pectins, raw fiber, betalains, ethanol

Introduction

Mexico is the country with the highest global diversity and abundance of prickly pear species, with approximately 80 recognized varieties, including green, yellow, criolla, red and xoconostle fruits; the State of Mexico led the national production in 2022 (SECAMPO, 2022). The *Opuntia* genus is widely consumed in the country and includes many species, among which are the nopal vegetable and prickly pear cactus. *Opuntia albicarpa* produces the fruit commonly known as “tuna,” characterized by juicy pulp and a low seed count (Lira-Ortiz *et al.*, 2014). In contrast, *Opuntia matudae* produces acidic fruits known as xoconostle, which contain a thick mesocarp and hard seeds located at the center (Gallegos-Vázquez *et al.*, 2012). These structural characteristics limit their industrial use, restricting xoconostle mostly to traditional culinary applications.

Pectin is a polysaccharide located in the middle lamella of plant cell walls (Muzzarelli *et al.*, 2012) that exhibits a variety of bioactive properties derived from its structural composition (Endress, 2011); for example, low-molecular-weight pectins, have been associated with potential anticancer activity in the colon, cardiovascular benefits, and reduced insulin levels (Almeida *et al.*, 2015). Previous studies have shown that xoconostle is a viable source of pectin for the food and pharmaceutical industries (Morales-Martínez *et al.*, 2018), as well as of betalains (Gengatharan *et al.*, 2015; Morales *et al.*, 2015) and fiber (Ramírez-Rodríguez *et al.*, 2020). However, these compounds have traditionally been obtained through independent processes, and no integrated approach has been implemented to recover multiple bioproducts simultaneously. Consequently, pigment-rich effluents generated during pectin extraction are typically discarded, and solid residues are also eliminated without further valorization.

A biorefinery is defined as a technological platform that enables the production of multiple value-added products (biopolymers, fibers, oils, etc.) from plant, animal, or microbial biomass (Sierra-Ibarra *et al.*, 2021). In this study, pectin was extracted from xoconostle, and the resulting by-products were subsequently used to obtain betalains, ethanol, and crude fiber, integrating these steps into a process under a biorefinery approach. In this way, the use of waste from the pectin industry is proposed, while simultaneously diversifying bioproduct generation and enhancing the value of xoconostle cultivation.

Materials and Methods

Pectin was extracted by acid hydrolysis, and the resulting residues were used for the recovery of betalains, ethanol, and crude fiber as described below.

Morphometric characterization

Xoconostle fruits were purchased from a local market in Mexico City. Length, equatorial diameter, and peel thickness were measured for the fruits. Peel, pulp, and seeds were separated to determine the proportion of these fractions relative to the total fruit.

Pectin extraction

The methodology of Zhang *et al* (2023) was used with minor modifications. The peel was chopped and ground in the presence of ethanol (70 °G.L.). The solids were sieved to remove the colored ethanol and subsequently washed to eliminate soluble compounds. All ethanol effluents were collected for betalain quantification and later distillation. The ground peel was dried to constant weight and designated as alcohol-insoluble residues (AIR). These solids were dispersed in acidified water at a 1:10 ratio (AIR:solvent), using citric acid solution (pH 3) because it is a more environmentally friendly acid compared to HCl, which is the most commonly used for this process. The dispersion was boiled for 30 minutes under constant agitation and centrifuged to obtain an extract rich in pectic substances, while the solid residue was dried and used to obtain raw fiber. Pectin was precipitated by mixing the extract with absolute ethanol (90 °G.L.) at a 1:1 ratio, forming a gel that was subsequently dried at room temperature. Dried pectin was ground and stored in amber containers prior to Fourier Transform Infrared Spectroscopy (FT-IR) analysis using a Bruker Tensor 27 spectrometer (Germany) equipped with attenuated total reflectance.

Total betalains and effluent distillation

Total betalains were quantified as the sum of betacyanins and betaxanthins using the method proposed by Soriano-Santos *et al* (2007) with slight modifications. Absorbance was measured at 600, 538, and 476 nm, using extinction coefficients of 1120 for betacyanins and 750 for betaxanthins.

Effluents were distilled at atmospheric pressure using a laboratory-scaled distillation unit, and 100 mL distillate fractions were collected periodically. Ethanol concentration was determined using an optical refractometer (PCNRF 080VV, Mettler Toledo, USA) and expressed in Gay-Lussac degrees (°G.L.). Distillation began at 78 °C and ended at 93 °C, when the vapor consisted predominantly of water.

Raw fiber

Raw fiber was obtained following the method described by Guerrero-Colín *et al* (2016). Solid residues were hydrolyzed with 1N HCl for 10 min under boiling conditions and washed with sodium hypochlorite. The remaining solids were dried to constant weight and quantified.

Results and Discussion

Morphometric characterization

Fruits of prickly pear xoconostle were separated into peel, pulp, and seeds. The peel was notably the thickest fraction, whereas the pulp and seeds occupied a minimal space in the central region (Figure 1). The average fruit mass was 74.7 ± 9.7 g, with the peel representing 86.92 % of the total weight (Table 1).

For physical characterization, length (6.05 ± 0.5 cm), equatorial diameter (4.67 ± 0.4 cm) and peel thickness (1.18 ± 0.1 cm) were measured, and their locations within the fruit are illustrated in Figure 1. Due to these morphological characteristics, the peel was the ideal substrate for pectin extraction.

Table 1. Proportion of the fractions of xoconostle fruits

	Peel	Pulp	Seed
g	64.92 ± 7.6	5.02 ± 1.4	4.76 ± 1.1
%	86.92 ± 2.5	6.66 ± 1.8	6.41 ± 1.5

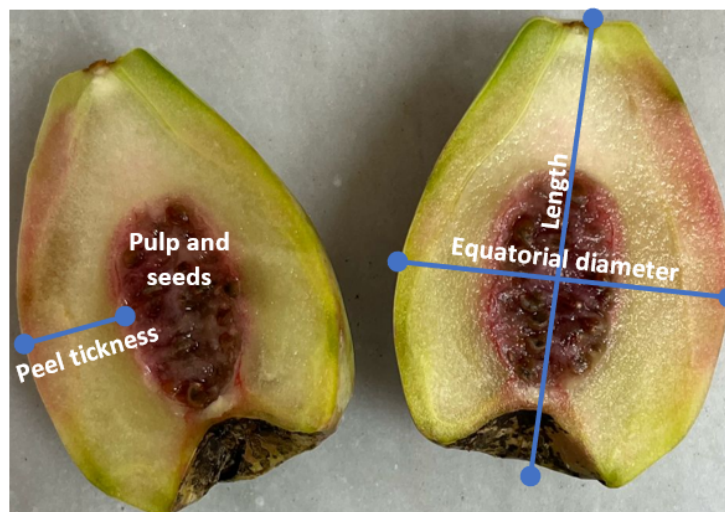


Figure 1. Morphometry of xoconostle fruits

Pectin extraction

Extraction process was carried out in several stages with the objective of obtaining an aqueous extract rich in pectic substances, from which the pectins were separated by insolubilization with ethanol.

It should be noted that an effluent with a high ethanol content was generated, which originated mainly from washing and precipitation, as shown in Figure 2 (b) and 2 (d).

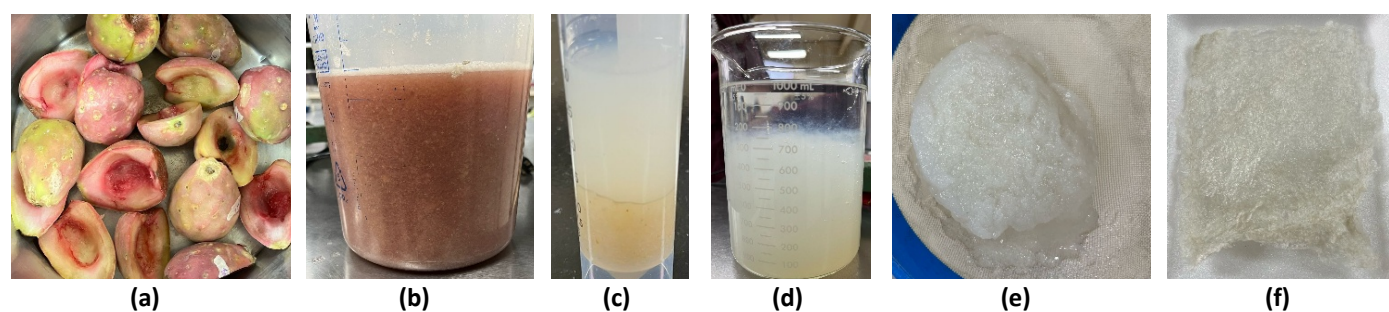


Figure 2. Main stages in pectin extraction: (a) Xoconostle peel, (b) Grinding and washing with ethanol to remove pigments, (c) Centrifugation to obtain the extract and solid residue, (d) Gel precipitation by ethanol addition, (e) Pectin gel isolation, and (f) Dried pectin for grinding

A pectin yield of 9.95 % (dry basis) was obtained, which is comparable to 10.46 % reported by Morales-Martínez *et al* (2018). FT-IR spectra (Figure 3) confirmed that the isolated material corresponded to pectin, showing characteristic functional groups and the “fingerprint region” between 1500 and 700 nm, one of the most important characteristics of pectins (Yang *et al.*, 2018). A prominent band at 3394 cm^{-1} indicated glucose units of the pectin backbone, while bands in 1737 and 1618 cm^{-1} corresponded to methoxyl groups.

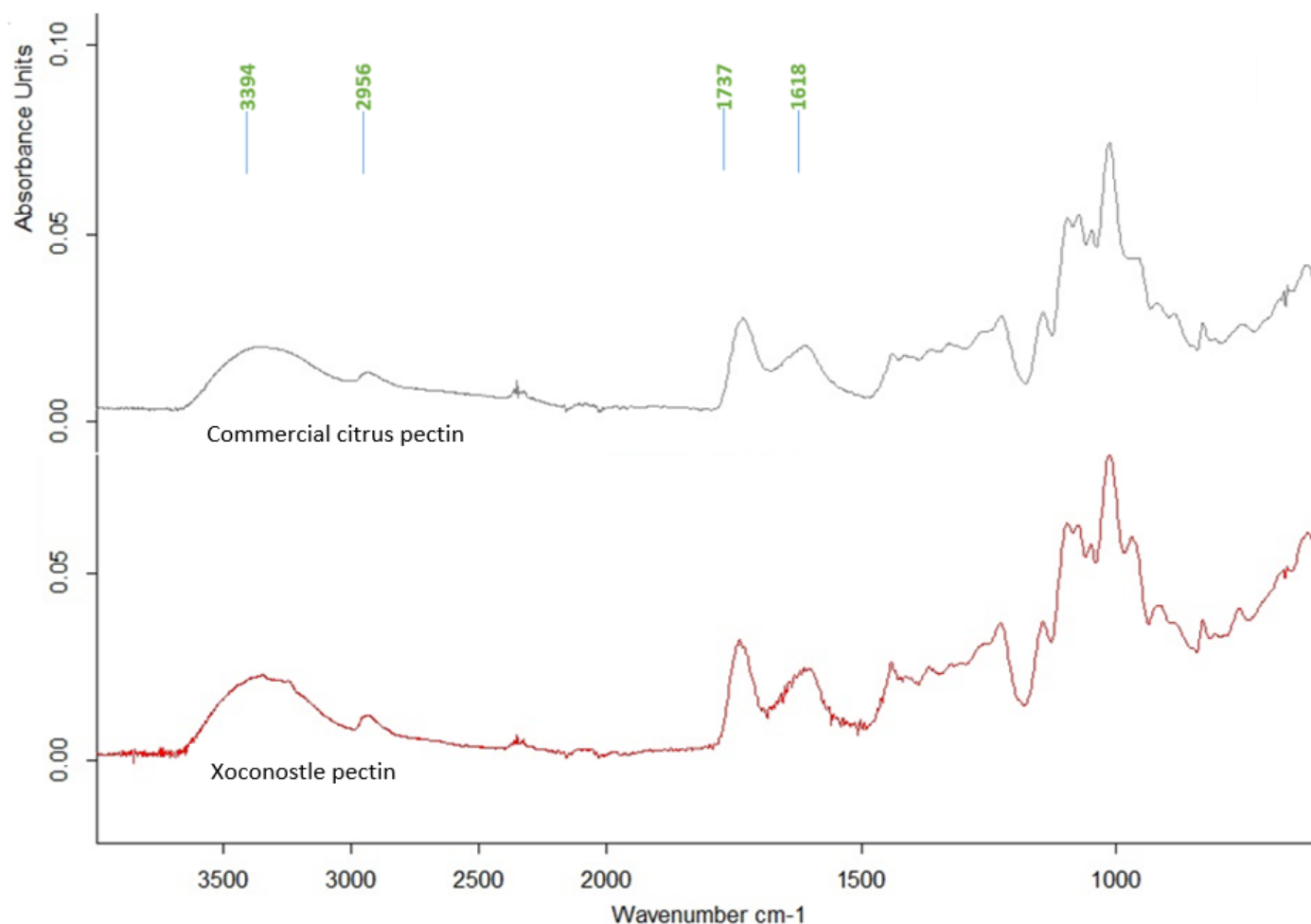


Figure 3. Spectrograms for xoconostle pectin compared with commercial citrus pectin

Total betalains and effluent distillation

Betalain yields were 0.746 g/mL for betacyanins and 1.275 g/mL for betaxanthins, totaling 2.021 g/mL. This demonstrates that process effluents represent a valuable source of natural pigments, thus contributing to the valorization of by-products through the extraction of this bioactive compound.

For effluents distillation, a complete temperature scan was carried out (from 20 °C to 93 °C), with vapor production beginning at 78 °C, approximately 25 min after starting heating as can be seen in Figure 4 (a). As the mixture lost ethanol, temperature increased until it stabilized at 93 °C. During this time mixture allowed ethanol elimination, leaving as a residue an effluent that mostly corresponded to water. This behavior is illustrated in Figure 4(b) where the distillate initially exhibited a concentration of 80 °G.L. which gradually decreased to 2 °G.L. as the vapor becomes predominantly aqueous. The total yield obtained (by mixing all 100 mL fractions) was 70 % with an average ethanol concentration of 65 °G.L. These results demonstrate that it is possible to recover high quality ethanol from peel washing effluents, which can then be reused in the pectin extraction process. It can also be observed that the second-order polynomial equation in Figure 4 (b) allows the prediction of ethanol concentration as a function of temperature.

Ethanol represents one of the major cost factors in pectin production, therefore distilling of effluents can significantly reduce production costs and promote solvent recycling.

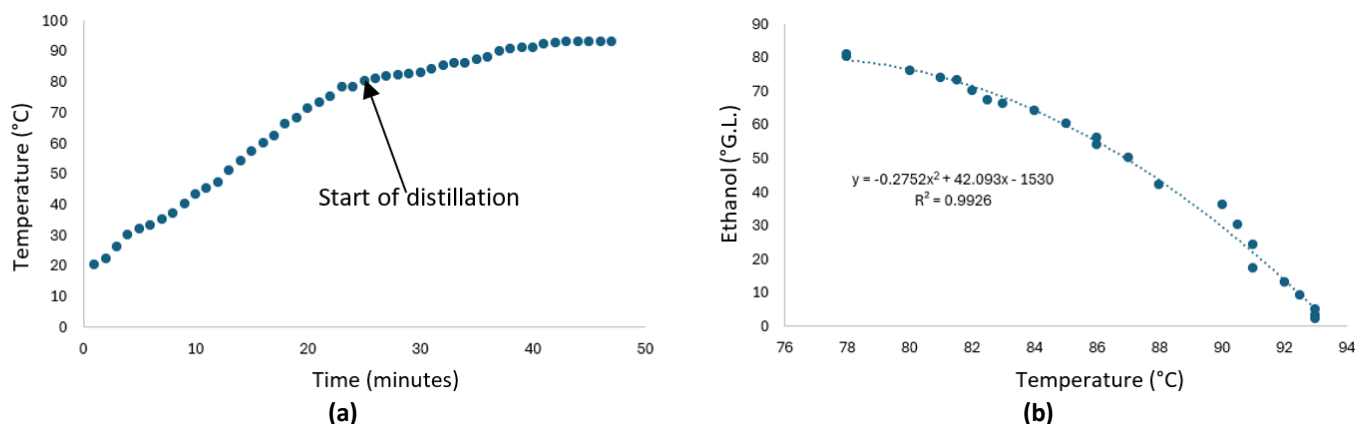


Figure 4. Distillation profiles: (a) temperature versus heating time, and (b) ethanol concentration as a function of temperature

Raw fiber

Raw fiber was isolated from residual solids obtained by centrifugation, as shown in Figure 2 (c). A yield of 72.3 ± 3.48 % of raw fiber was obtained, because starch, protein and part of the lignin were solubilized, leaving mainly non-digestible components such as cellulose, hemicellulose and residual lignin (AOAC 7.068, 1995). Crude fiber has important applications in food industry (e.g., as a dietary fiber source in cookies and breads) and in biotechnology sector as a fermentation substrate for metabolite production.

Proposal for a biorefinery

Pectin extraction involves several stages that generate by-products with potential value for bioproduct recovery. A proposal for the integrated utilization of products and by-products from this industry is illustrated in Figure 5.

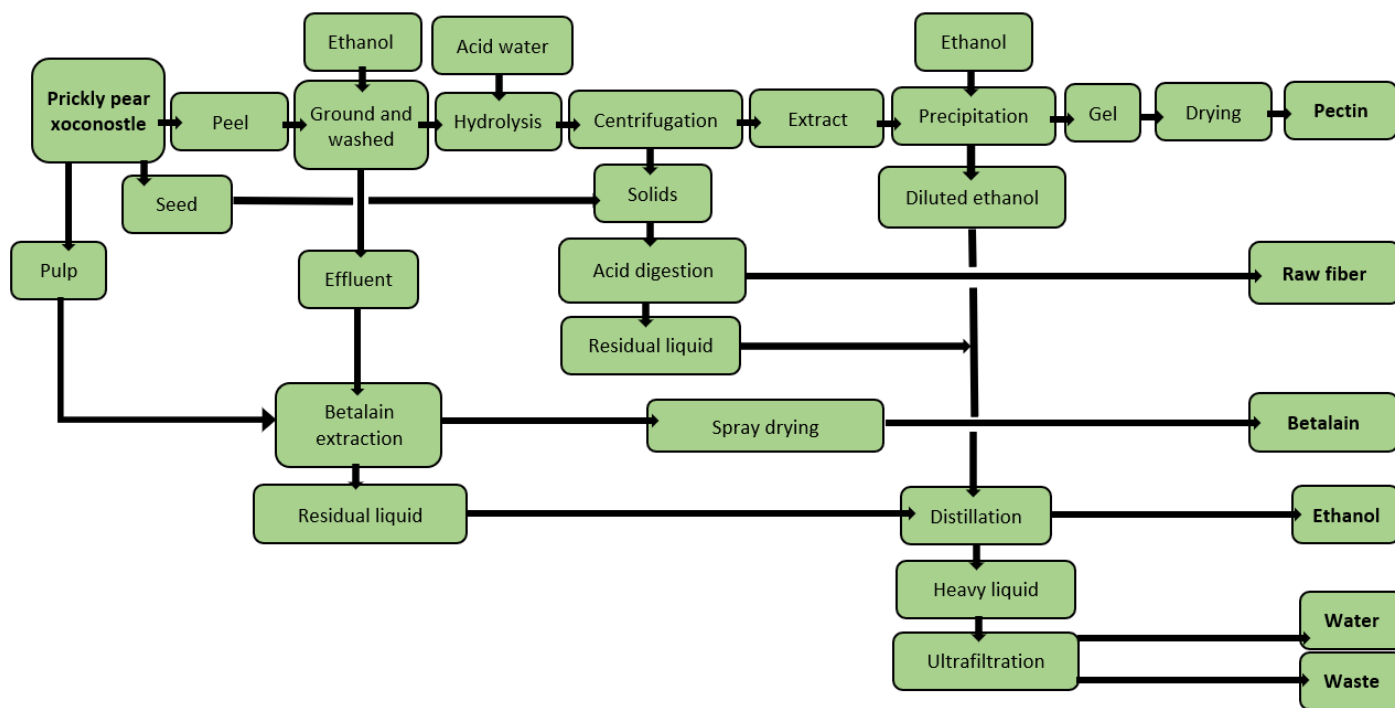


Figure 5. Proposed biorefinery scheme for the extraction of pectin and crude fiber, betalains recovery, and ethanol and water recycling

As shown in (Figure 5), pectin is the main target product. The washing effluent undergoes betalain recovery and is subsequently sent to distillation together with the ethanol used for gel precipitation. The solids obtained after centrifugation can be combined with seeds for raw fiber production. In addition, it is proposed that the remaining liquid fraction (mainly pigmented water) be treated through membrane ultrafiltration to recover water for reuse in the process. In this way, a single technological unit integrates multiple valorization pathways, allowing pectin extraction to evolve towards a more sustainable and diversified biorefinery approach.

Conclusions

Peels of xoconostle prickly pear fruit were shown to be an important source for the extraction of pectin with properties comparable to those of commercial pectin.

The pectin production process generates several by-products, including water-ethanol mixtures, pigment-rich leachates, and solid shell residues, from which betalains, crude fiber, and recoverable ethanol were obtained.

The valorization of pectin extraction waste within a biorefinery approach contributes to the development of a more sustainable and environmentally friendly industry, promoting the transition of pectin production toward a circular and resource-efficient process.

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Author contributions: L.D.T.-G. and A.B.-R: experimental process and data collection; E.F.-G: analysis; L.L.-A: project administration; G.G.-C: provide materials and editing; J.P.-R: supervision and writing.

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